भारतीय मानक
Indian Standard

IS 543 : 2018

बिनौले का तेल — विशिष्टि

(तीसरा पुनरीक्षण)

Cottonseed Oil — Specification

(Third Revision)

ICS 67.200

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May 2018 **Price Group 5**

Oils and Oilseeds Sectional Committee, FAD 13

FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Unlike coconut, groundnut, sesame and mustard oils, raw or unrefined cottonseed oil is not consumed in India for edible purpose. It is used as a salad oil after suitable refining and deodorizing, and is also used in the manufacture of hydrogenated vegetable oil products.

This standard was originally published in 1954. In its first revision issued in1966, raw grade oil and an additional grade under washed oil were introduced. As the standard covered only the expressed type of oil and since there was an urgent need for the formulation of an Indian Standard for the solvent-extracted type, IS 3472 : 1960 was issued an emergency standard. After having decided to normalise the emergency standard, the need for rationalizing the various grades of the oil covered in the standards was keenly felt. Hence the second revision of IS 54 was prepared amalgamating its earlier revisions and IS 3472 : 1960. The second revision was later amended to introduce scheme for labelling environment friendly products to be known as ECO-Mark at the instance of the Ministry of Environment and Forests (MoEF).

This revision has been carried out to harmonize the standard with *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Vegetable Oils Grading and Marking Rules*, 1955.

In this revision the following major changes have been made:

a) The nomenclatures of the different grades of mustard oil have been changed;
b) Solvent extracted semi refined grade has been removed;
c) Grades used exclusively for industrial purpose have been removed;
d) The limit of aflatoxin has been prescribed for non-ECO marked edible oils also;
e) Aflatoxin is determined using High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) instead of Thin Layer Chromatography (TLC) prescribed earlier;
f) The colour of refined oil is determined using 100 mm cell on the Lovibond scale instead of ¼ inch cell prescribed earlier; and
g) Bellier trubidity temperature and limit of hexane has been incorporated to align with *Food Safety and Standards (Food Product Standards and Food Additives) Regulation*, 2011.

In the formulation of this standard, due consideration has been given to *Food Safety and Standards Act*, 2006 and Regulations framed thereunder; *Legal Metrology Act*, 2009 and Rules framed thereunder and the *Essential Commodities Act*, 1955. However, this standard is subject to restrictions imposed under these, wherever applicable.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

COTTONSEED OIL — SPECIFICATION

(Third Revision)

1 SCOPE

This standard prescribes requirements and methods of sampling and test for cottonseed oil used for edible purposes and for manufacture of refined oil and *Vanaspati*.

2 REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
548	Methods of sampling and test for oils and fats:
(Part 1) : 1964	Methods of sampling, physical and chemical tests (*revised*)
(Part 2) : 1976	Purity tests (*third revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
IS 1448 [P: 21] : 2012 /ISO 2719 : 2002	Method of test for petroleum and its products: [P : 21] Determination of flash point — Pensky Martens close cup method (*third revision*)
1699 : 1995	Methods of sampling and test for food colours (*second revision*)
2619 : 1993	Glass Beakers — Specification (*second revision*)
3470 : 2002	Hexane, food grade — Specification (*first revision*)
10142 : 1999	Polystyrene (crystal and high impact) for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification (*first revision*)
10146 : 1982	Specification for polyethylene for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
10151 : 2014	Specification for polyvinyl chloride (PVC) and its copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
10325 : 2000	Square tins — 15 kg/litre for ghee, *VANASPATI*, edible oils and bakery shortenings — Specification (*second revision*)
10339 : 2000	Ghee, *VANASPATI*, edible oil tins up to 10 kg/litre capacity — Specification (*second revision*)
10910 : 1984	Specification for polypropylene and its copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
11434 : 1985	Specification for ionomer resins for its safe use in contact with foodstuffs, pharmaceutical and drinking water
11704 : 1986	Specification for ethylene acrylic acid (EAA) copolymers for their safe use in contact with foodstuffs, pharmaceuticals and drinking water
12247 : 1988	Specification for nylon-6 polymer for its safe use in contact with foodstuffs pharmaceuticals and drinking water
12252 : 1987	Specification for polyalkylene terephthalates (PET & PBT) for their safe use in contact with foodstuffs, pharmaceuticals and drinking water
13576 : 1992	Ethylene menthacrylic acid (EMAA) copolymers and terpolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification
13601 : 1993	Ethylene vinyl acetate (EVA) copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification
IS/ISO 14718 : 1998	Animal feedings stuffs — Determination of aflatoxin B1 content of mixed feeding stuffs — Method using high performance liquid chromatography

3 DEFINITION

For the purpose of this standard, the definitions given in **2** of IS 548 (Part 1) and also the following shall apply.

3.1 Refined Cottonseed Oil — Refined cottonseed oil obtained by expression or solvent extraction of cottonseed oil bearing material, deacidified either with alkali or by physical refining or by miscella refining using food grade solvents followed by bleaching with

Table 1 Requirements for Cottonseed Oil

(*Clause* 5.7)

Sl No.	Characteristic	Requirement				Method of Test, Ref to
		Expressed		Solvent-extracted		
		Refined Grade	Washed Grade	Refined Grade	Raw Grade	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i)	Moisture and insoluble impurities, percent by weight, *Max*	0.10	0.10	0.10	0.75	**5** and **6** of IS 548 (Part 1)
ii)	Colour on the Lovibond scale, expressed as ($Y + 10R$), not deeper than	20[1)]	35[2)]	30[1)]	—	**13** of IS 548 (Part 1)
iii)	Refractive index at 40°C	1.463 0 to 1.466 0				**10** of IS 548 (Part 1)
iv)	Specific gravity at 30ºC/30ºC	0.910 to 0.920				**11** of IS 548 (Part 1)
v)	Saponification value	190 to 198				**15** of IS 548 (Part 1)
vi)	Iodine value (Wijs)	98 to 112				**14** of IS 548 (Part 1)
vii)	Bellier turbidity temperature, °C	19 to 21				**13** of IS 548 (Part 2)
viii)	Acid value, *Max*	0.5	0.5	0.5	10.0	**7** of IS 548 (Part 1)
ix)	Unsaponifiable matter, percent by mass, *Max*	1.5	1.5	1.5	2.0	**8** of IS 548 (Part 1)
x)	Flash point (Pensky-Martens), closed, ºC, *Min*	—	—	250	100	IS 1448 [P : 21]
xi)	Hexane, ppm, *Max*	—	—	5.00	5.00	Annex B
xii)	Refining loss, percent by weight, *Max*	—	—	—	15.0	Annex C

[1)] in a 100 mm cell

[2)] in a 10 mm cell

absorbent earth and/or carbon and deodorized with steam.

4 TYPES AND GRADES

4.1 The material shall be of the following types and grades:

a) *Expressed type:*
   1) Refined Grade, and
   2) Washed Grade.

b) *Solvent-extracted type:*
   1) Refined Grade, and
   2) Raw Grade.

4.1.1 Refined grades of both expressed and solvent extracted types are suitable for direct edible consumption.

4.1.2 Washed grade of expressed type and raw grade of the solvent-extracted type are suitable for making refined oil and *Vanaspati* and not for direct edible consumption.

5 REQUIREMENTS

5.1 Description

The material shall be obtained from good quality cottonseed cake or from clean sound cottonseed kernel from *Gossypium* spicies, fam. Malvaceae. by a process of solvent extraction or from the cottonseed by a process of expression.

5.1.1 Solvent extracted type cottonseed oil shall be obtained from the oleaginous material using solvent hexane conforming to IS 3470.

5.2 The material shall be clear and free from rancidity, adulterants, sediment, suspended and other foreign matter, separated water and added colouring and flavouring substances.

5.2.1 The clarity of the material shall be judged by the absence of turbidity after keeping the filtered sample at 30ºC for 24 h.

5.3 Oils shall be free from non-edible oils and adulterants when tested in accordance with **9**, **10, 11, 12, 14, 15** and **16** of IS 548 (Part 2).

5.4 Oils shall not contain aflatoxin, more than 30 µg/kg, when tested by the method prescribed in IS/ISO 14718 or as prescribed in Annex A.

5.5 Metal contaminants and pesticide residues shall not exceed the tolerance limits as prescribed in the *Food Safety and Standards (Contaminants, Toxins and Residues) Regulations*, 2011.

5.6 Only permitted antioxidants and antioxidant synergists not exceeding the quantities specified against each as prescribed under the *Food Safety and Standards (Contaminants, Toxins and Residues) Regulations*, 2011, may be used, if required.

5.7 The material shall also comply with the requirements given in Table 1.

5.8 Optional Requirements for ECO-Mark

5.8.1 The product shall conform to the requirements of quality as given in **5.1** to **5.7**.

5.8.1.1 The manufacturers shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the norms laid down under the *Water* (*Prevention and Control of Pollution*) *Act*, 1974; *Air* (*Prevention and Control of Pollution*) *Act*, 1981; *Water* (*Prevention and Control of Pollution*) *Cess Act*, 1977 respectively, along with the authorization, if required, under the *Environment* (*Protection*) *Act*, 1986, while applying for ECO BIS Mark.

5.8.1.2 The product shall not contain aflatoxin, more than 5 µg/kg, when tested by the method prescribed in IS/ISO 14718 or as prescribed in Annex A.

5.8.1.3 The product shall not contain any of the toxic metals in excess of the quantities prescribed in Table 2.

Table 2 Limits for Toxic Metals

(*Clause* 5.8.1.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Lead, mg/kg, Max	0.5	**15** of IS 1699
ii)	Arsenic, mg/kg, Max	0.5	do
iii)	Cadmium, mg/kg, Max	1.0	do
iv)	Mercury (total) mg/kg, Max	0.25	do

6 PACKING

6.1 The material shall be supplied in suitable well-closed tin or plastic containers, as agreed to between the purchaser and the supplier. Tin or plastic containers once used, shall not be re-used for packaging of edible oils and fats.

Containers made of plastic materials shall be as per IS 10142 or IS 10146 or IS 10151 or IS 10910 or IS 11434 or IS 11704 or IS 12247 or IS 12252 or IS 13601 or IS 13576.

Containers made of tin shall be as per IS 10325 or IS 10339.

6.1.1 For ECO-Mark, the product shall be packed in such packages which are made from recyclable (that is which can be re-processed to manufacture any useful product) or biodegradable materials.

6.2 Types and grades not suitable for direct edible consumption shall not be packed in consumer packs.

7 MARKING

7.1 The containers shall be marked in English or Hindi in *Devnagri* script with the following information:

a) Name, trade name, type and grade of the oil;
b) Name and business particulars of the manufacturer;
c) Net quantity of the contents in the container;
d) Batch number, month and year of manufacture;
e) Free from Argemone Oil;
f) *Nutritional information* — Nutritional information or nutritional facts per 100 g or 100 ml or per serving of the product shall be given on the label containing the following:
   1) Energy value, in kcal;
   2) Amounts of protein, carbohydrate (specify quantity of sugar) and fat in gram (g) or ml;
   3) Amount of any other nutrient for which a nutrition or health claim is made:

   Provided that where a claim is made regarding the amount or type of fatty acids or the amount of cholesterol, the amount of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, in gram (g), and cholesterol, in milligram (mg), shall be declared, and the amount of trans fatty acid, in gram (g), shall be declared in addition to the other requirement stipulated above.
g) Any other requirement as stipulated under *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Legal Metrology Act*, 2009 and rules framed thereunder.

7.2 The container of imported edible oil shall also bear the word, 'Imported', as prefix to type and grade of oil.

7.3 In addition in the case of the types and grades which are not suitable for direct edible consumption (*see* **4.1.2**), the following information shall be suitably marked, either printed on the label affixed to the container or lithographed or stencilled thereon with indelible ink, in a type size of not less than 50 mm:

'NOT FOR DIRECT EDIBLE CONSUMPTION'

7.4 The package, label or the advertisement of edible oils and fats shall not use the expressions 'Super-Refined', 'Extra-Refined', 'Micro-Refined', 'Double-Refined', 'Ultra-Refined', 'Anti-Cholesterol', 'Cholesterol Fighter', 'Soothing to Heart', 'Cholesterol Friendly', 'Saturated Fat Free' or such other expressions which are an exaggeration of the quality of the product.

7.5 For ECO-Mark the containers shall be marked with the following:

a) List of identified critical ingredients in descending order of quantity, percent by mass, which shall include 'made from cottonseed oil';

b) The brief criteria for which the product has been labelled for ECO-Mark; and

c) Shelf life of the product.

7.6 BIS Certification Marking

The product may also be marked with the Standard Mark.

7.6.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

7.7 ECO-Mark

The product may also be marked with the ECO-Mark, the details of which may be obtained from Bureau of Indian Standards.

8 SAMPLING

8.1 Representative samples of the material shall be drawn as given in **3** of IS 548 (Part 1).

ANNEX A

(*Clauses* 5.4 *and* 5.8.1.2)

DETERMINATION OF TOTAL AFLATOXIN BY ELISA

A-1 PRINCIPLE

Antibodies specific to aflatoxins B1, B2 and G1 are immobilized on the filter, and toxin (aflatoxin B1) is labelled with an enzyme (horseradish peroxidase). Binding of toxin-enzyme conjugate by immobilized antibodies is inhibited by addition of free toxin present in the test sample. Bound enzyme catalyses oxidation of substrate to form a blue complex. Development of colour indicates that the test sample contains aflatoxin.

A-2 APPARATUS

A-2.1 Antibody Coated Solid Support.

A-2.2 Aflatoxin Enzyme Conjugate

A-2.3 High Speed Blender

A-2.4 Variable 100-1 000 ìl Micropipettes

A-2.5 Glass Culture Tubes

A-2.6 Filters

A-2.7 Timer

A-2.8 Silicon Carbide Boiling Chips

A-3 REAGENTS

A-3.1 Wash Solution-Phosphate Buffered Saline Solution — Dissolve 0.23 g $NaH_2PO_4.H_2O$, 1.95 g $K_2HPO_4.3H_2O$, 8.70 g NaCl, 0.125 ml Tween 20 and 10 mg thimerosal in 900 ml H_2O, adjust *p*H to 7.2 and dilute to 1 l.

A-3.2 Buffer — 0.1 percent Bovine serum albumin in phosphate buffer saline solution containing 0.05 percent thimerosal.

A-3.3 Substrate Solution A, tetramethylbenzidine (TMB), (0.4 g/l H_2O), *p*H 8.3.

A-3.4 Substrate Solution B, hydrogen peroxide (0.02 percent H_2O_2 in 0.13 percent aq. Citric acid solution, *p*H 3.0).

A-3.5 Methanol

A-3.6 Hexane

A-3.7 Chloroform

A-3.8 NaH_2PO_4

A-3.9 K_2HPO_4

A-3.10 NaCl

A-3.11 Tween 20

A-3.12 Bovine Serum Albumin

A-4 PROCEDURE

A-4.1 Preparation of Sample

A-4.1.1 Weigh 50 g of sample into blender jar.

A-4.1.2 Mix with 250 ml of 55 percent methanol and 45 percent water (*see* IS 1070).

A-4.1.3 Mix 100 ml hexane and blend for 1 min at high speed.

A-4.1.4 Filter mixture and recover filtrate.

A-4.1.5 Leave for 5 min and remove the lower phase containing methanol water (*see* **A-4.1.2**).

A-4.2 Testing

A-4.2.1 Bring all reagents at room temperature (20-23°C).

A-4.2.2 Prepare fresh substrate in small culture tubes by mixing 500 µl substrate solution A with 500 µl substrate solution B for each reaction sites used.

A-4.2.3 Add 100 µl test extract to 200 µl buffer (*see* **A-3.2**).

A-4.2.4 Thoroughly mix the diluted test extract and apply 100 µl diluted test extract to the centre of membrane. Using timer, wait for 1 min.

A-4.2.5 Apply 100 µl (2 drops) enzyme solution to the centre of membrane. Using timer, wait for 1 min.

A-4.2.6 Wash with 1.5 ml (30 drops) wash solution added drop wise.

A-4.2.7 Add the entire content of the substrate solution 1.0 ml from each test tube to each reaction site. Wait 1 min and immediately observe site (centre of cup) for blue colour development (negative) or no colour development (positive).

A-4.3 Interpretation of Results

A-4.3.1 Observe the reaction site (centre of the cup) for a blue colour or no colour development at exactly 1 min after adding the substrate A and B mixture (*see* **A-3.3** and **A-3.4**).

Negative — If the reaction site (centre of the cup) turns light blue or darker, test sample contains total aflatoxin B1, B2 and G1.

Positive — If no blue colour is observed in the reaction site (centre of cup) and reaction site remains completely white (no colour change) for at least 1 min, the test sample contains aflatoxin B1, B2 and G1.

ANNEX B

[*Table* 1, *Sl No.* (xi)]

DETERMINATION OF HEXANE RESIDUES IN OILS AND FATS

B-1 PRINCIPLE

The residual hexane content is the quantity of volatile hydrocarbons remaining in the fats and oils following processing involving the use of solvents. The volatile hydrocarbons are desorbed by heating the sample at 80°C in a closed vessel after addition of an internal standard. After determination of a calibration factor, hydrocarbons in the head space are determination of a calibration factor, hydrocarbons in the head space are determined by gas chromatography using packed or capillary columns. Results are expressed as hexane in mg/kg (or ppm). The method is applicable to the determination of 'free' volatile hydrocarbons expressed in terms of hexane remaining in animal and vegetable fats and oils after extraction with hydrocarbon based solvents. It is suitable for determination of quantities of hexane between 10 and 1 500 mg/kg in fats and oils.

B-2 APPARATUS

B-2.1 Gas Chromatograph

Gas chromatograph having,

a) thermostatic column capable of maintaining the desired column temperature with in ± 1°C;
b) sample inlet system, separately thermostated which can be maintained at a minimum temperature of 100°C. If a capillary column is used, the inlet system must be capable of a 1/100 split injection. For serial analysis a headspace gas chromatograph with automatic sample injection and tempering bath is satisfactory; and
c) flame ionization detector which can be separately thermostated and maintained at a minimum of 100°C.

B-2.2 Recorder

If a recorder trace is to be used for calculating the composition of the samples analyzed, an electronic recorder of high precision is required or else use electronic integrator (*see* **B-2.3**)

B-2.3 Electronic Integrator, which permits rapid and accurate calculations.

B-2.4 Chromatographic Column, either packed or capillary column with the following minimum requirements:

a) *Packed column* — stainless steel or glass,

approx 2 m long and 3.175 mm internal diameter with acid washed and silanized diatomaceous earth, 150-180 mm particle size (80-100 mesh Chromosorb WAW is suitable), stationery phase — squalene consisting of 10 percent of packing.

b) *Capillary column* — glass or fused silica approx 30 m long and 0.3 mm internal diameter.

c) Stationery phase — Methyl polysiloxane (film thickness 0.2 mm).

B-2.5 Syringe — 1 ml, 10 m1, 1 000 ml capacity, gas tight.

B-2.6 Septum Vial — 20 ml capacity.

B-2.7 Septa and Aluminium Caps Suitable for Septum Vials Together with Crimping Pliers

The septa must be resistant to oils and solvents (butyl rubber or red rubber is recommended.)

B-2.8 Tongs, suitable for holding septum vials.

B-2.9 Heating Bath, with clamps for holding septum vials, thermostatically regulated and capable of maintaining a temperature of 80°C. For continuous operation glycerol is recommended as heating liquid.

B-2.10 Shaking Machine

B-3 REAGENTS

B-3.1 Gases

a) *Carrier* — Helium (preferred for better resolution) or Nitrogen 99.99 percent pure, dried and containing a maximum of 10 mg O_2/kg.

b) *Flame Ionization Detector* — Hydrogen, minimum purity 99.95 percent, air or oxygen, dry, hydrocarbon free (less than 2 ppm hydrocarbon equivalent to CH_4).

B-3.2 Technical Hexane or Light Petroleum, with a composition similar to that used in industrial extraction or failing these *n*-hexane. For calibration, technical extraction hexane is preferred.

B-3.3 *n*-Heptane — (internal standard) analytical reagent grade.

B-3.4 Vegetable Oil — Solvent free, freshly refined and deodorized. The oil is to be used for calibration and should be of a similar nature as the sample. It should be free from extraction solvent (less than 0.01 percent).

B-4 SAMPLING AND SAMPLE STORAGE

It is essential that loss of solvent from the sample be prevented. The laboratory sample should be in a completely sealed condition and stored at 4°C. Plastic containers should not be used. Sample analysis should be carried out immediately when the sample container is opened.

B-5 GC OPERATING CONDITIONS

Carrier gas flow depends on the carrier gas and the type of column being used for analysis and should be optimized accordingly. The flow of hydrogen and air or oxygen to the FID should be optimized according to the manufacturer's recommendation. Injector and detector temperatures should be set at about 120°C. The column should be maintained at 40°C.

B-6 PROCEDURE

B-6.1 Determination of the Calibration Factor

Weigh to the nearest 0.01 g, 5 g of solvent free vegetable oil (*see* **B-3.4**) into each of the 7 septum vials. Seal each vial with a septum and cap. By means of a syringe add technical Hexane to 6 of the seven vials (in the vial with no added solvent is the blank) according to the following table:

ml/5g	0.5	1	2	4	7	10
mg/100g	67	134	268	536	938	1 340

One vial remains without the addition of solvent.

If *n*-hexane is used for calibration the following table applies

ml/5g	0.5	1	2	4	7	10
mg/100g	66	132	264	528	924	1 320

Shake the 6 vials containing the solvent in the shaking machine vigorously for 1 h. Using the syringe add 5 ml of internal standard (*see* **B-3.3**) to each of the 7 vials. Successively immerse the vials upto the neck in the heating bath at 80°C at intervals of approx 15 min. This time interval depends on the duration of the GC analysis which is complete on the elution of the internal standard (*n*-heptane). The samples must be placed in the heating at intervals such that each sample is tempered for exactly 60 min.

Warm the gas tight syringe to 60°C. After tempering at 80°C for exactly 60 min and without removing the vial from the heating bath, use the gas tight syringe and withdraw through the septum 1 000 m1(1 ml) of the head space above the oil. inject immediately into the gas chromatograph. For each of the vial containing added solvent a calibration factor *F* may be determined by the following formula.

$$F = \frac{C_S \times A_1}{(A_H - A_B - A_1) \times C_1}$$

where

A_H = total peak area of solvent hydrocarbons including the area of internal standard present in the spiked oil. For identification purposes a typical chromatogram of solvent composition should be obtained. Hydrocarbons which usually make up the technical hexane are 2 methyl pentane, 3 methyl pentane, methyl cyclo pentane, cyclohexane etc. Do not include peaks due to oxidation products which may be present in significant amounts.

A_B = peak area of the solvent hydrocarbons present in the oil to which solvent has not been added (blank) less the peak are of the internal standard.

A_1 = peak area corresponding to the internal standard in the spiked samples.

C_1 = quantity of the internal standard added expressed in mg/kg of the oil.

C_s = quantity of technical hexane added to the oil present in the vial expressed in mg/kg of the oil.

Express the results to the third decimal place.

Calibration factors of the six standards should be approximately the same. The mean calibration factor should be 0.45 if *n*-heptane is used and 0.57, if cyclohexane is used.

The factor (F) so evaluated can be used for determining vial quantities of hexane less than 60 mg/kg. If the value of F found for the vial containing 0.5 ml of hexane is significantly below the mean value, this deviation is probably due to difficulty in introducing exactly 0.5 ml and this determination must be either eliminated or repeated. For quantities of hexane between 10 and 20 mg/kg it is better to prepare calibration standards by adding 2 ml of internal standard instead of 0.5 ml.

B-6.2 Sample Analysis

Weigh to the nearest 0.01 g, 5 g of the test sample into a septum vial as quickly as possible and close immediately with a septum and cap. Using a syringe add through the septum exactly 5ml of the internal standard. Shake vigorously by hand for about 1 min and then immerse the vial upto the neck in the heating bath. At 80°C for exactly 60 min. Warm the gas tight syringe to 60°C. After tempering at 80°C for exactly 60 min use the gas tight syringe and take from the vial without removing it from the bath 1 000 ml (1 ml) of the head space above the sample. Immediately inject into the gas chromatograph. Carry out two determinations in rapid succession on each sample.

B-8 CALCULATION

The residual solvent expressed in mg/kg (ppm) is given by the following formula:

$$W = \frac{(A_H - A_1) \times F \times C_1}{A_1}$$

where

A_H = total peak area of solvent hydrocarbons including the area of internal standard. Hydrocarbons which usually make up the technical solvents are 2 methyl pentane, 3 methyl pentane, methyl cyclopentane, cyclohexane etc. Do not include peaks due to the oxidation products. Some of these products may be present in significant amount.

A_1 = peak area corresponding to internal standard in the sample.

C_1 = quantity of the internal standard added in mg/kg.

NOTE — For an addition of 5 ml of heptane/5 g of sample C_1 = 680 mg/ kg and C_1 = 750 mg/kg, if cyclohexane is used.

F = calibration factor obtained in procedure

Report as the final result the mean of the results of two determinations.

ANNEX C

[*Table* 1, *Sl No.* (xii)]

DETERMINATION OF REFINING LOSS

C-1 REFINING LOSS

This method determines the loss of free fatty acids, oil and impurities when the sample is treated with alkali solutions under the specific conditions of the test.

C-2 APPARATUS

C-2.1 Balance — Torsion type or equivalent, capacity 1 000 g and sensitive to 0.1 g.

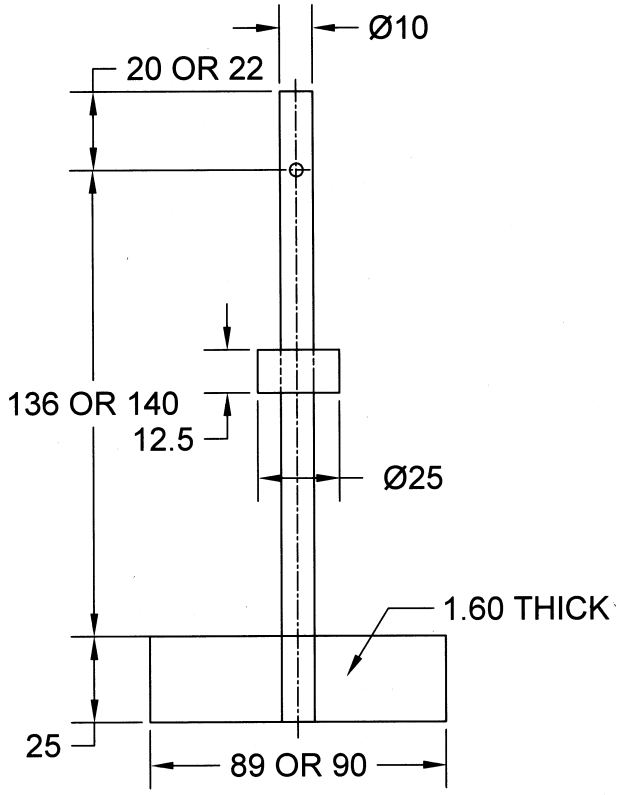
C-2.2 Refining Cups — 1 000 ml medium type glass beaker. (*see* IS 2619), or seamless stainless steel cups of equivalent size.

C-2.3 Refining Apparatus

The assembly shall consist of flat bottomed metal vassal of about 4 l capacity to serve as the bath for the refining cups. The bath shall be provided with cup supports so arranged as to hold the cups in a fixed position. The bath shall have suitable hot and cold water connections and adequate heating arrangement so that the change of temperature from cold to hot, as required, may be attained in a minute. Separate baths may also be used for the hot and cold water, if desired.

The water in the bath shall be kept at a uniform temperature by suitable agitation and the level shall be at least as high as that of the oil and alkali within the refining cups.

The paddles run by a suitable electric motor with a variable speed control shall be provided for the agitation of the samples in the refining cups. The paddles for this purpose shall be of stainless steel or nickel-plated copper or brass in accordance with the dimensions shown in Fig. 1. The paddles should be rigid enough 10 that their bottom is 6 mm above the bottom of the refining cups at all times during the test. Agitation of the samples is required at 70 ± 5 rpm and 250 ± 10 rpm.



All dimensions in millimetres.

FIG. 1 PADDLE FOR REFINING APPARATUS

NOTE — The slow speed or 70 rpm may also be achieved by hand rotation of the stirrer. The highest speed of 250 rpm may be visually checked by the formation of a conical depression of 4 cm depth extending to about less than half the height of the fluid. Accurate control of the speeds required should, however, be done by placing a white dot of paint on the stirrer shaft and counting the rotation visually against the time with the help of Stopwatch.

C-3 REAGENTS

C-3.1 Standard Sodium Hydroxide solution, 0.1 N.

C-3.2 Sodium Hydroxide Solution (20°Be or about 15 Percent w/w)

This shall be of accurately known sodium hydroxide content, free from carbonate and other impurities, and prepared as follows:

Add 750 g of distilled water to 1 000 g of pure, dry, solid sodium hydroxide. Heat on the steam-bath with occasional stirring for at least 3 h. Cool and allow to settle for 24 to 48 h, keeping the vessel covered to exclude air. During cooling, a portion of the sodium hydroxide which was dissolved in the hot solution crystallize and precipitates. If the crystals do not separates the solution was not supersaturated and may not be satisfactory. If properly prepared as described, the solution contains no measurable quantity of carbonate or other impurities. Allow to settle until clear and then carefully decant. Filter through filter paper or asbestos, if the solution is not-perfectly clear and protect from the air during filtration to prevent the absorption or carbon dioxide. This solution is then diluted with previously boiled and cooled distilled water to 20° Be (14·36 percent by weight) concentration. The actual strength of tile solution shall be determined by weighing and titrating with standard acid. Specific gravity and Baume test are not sufficiently accurate for the purpose of this test.

C-4 PREPARATION OF SAMPLE

The sample container shall be vigorously shaken and the ample thoroughly mixed in order to incorporate and uniformly distribute meal or other sediment. If the oil is cold, heat to 20°C before shaking. Inspect the inside of the can to make sure that no sediment remains clinging to the sides or bottom. If any sediment is found, remove it completely (cut the can open, if necessary) and incorporate thoroughly with the oil. The uniform incorporation and distribution of settlings and suspended matter are very significant in determining the accuracy of the final refining loss.

'*Caution* — SAMPLES SHALL NOT BE ALLOWED TO COME IN CONTACT WITH COPPER SINCE. THE COLOUR MAY THEREBY BE AFFECTED'

C-5 PROCEDURE

Determine the acid value as given in **7** of IS 548 (Part 1).

Weigh 500 g of thoroughly mixed sample into the refining cup and place it in the water-bath. Remove any persistent foam in the oil before adjusting the final weight. Fill the water-bath to the specified height with tap water.

Start the stirrer and run it at the high speed of 250 rpm and add as quickly as possible the calculated amount of alkali (*see* Note). Continue the high speed agitation for 45 min.

NOTE — The amount of sodium hydroxide in gram required for 100 gram of this oil is: $\frac{\text{Acid Value}}{8.73}$ 0.77

Change the agitation to slow speed of 70 rpm and increase the water-bath temperature to 63°C to 67°C. This temperature change should be completed in one minute. This may allow be done by adding 2.5 l. of boiling water to the outer vessel. Ensure that the level of water in the outer vessel is 5 cm above the oil level inside the cup. Continue stirring and maintain the temperature for 12 min.

Continue the agitation, remove the stirrer, drain thoroughly against one edge of the stirrer and allow the refined oil in the cup to settle overnight.

Weigh the cup and the contents and deduct this weight from the total weight at start to obtain evaporation loss.

Decant the refined oil in another tared refining cup and drain the soap stock for 30 min by inverting the soap stock cup in the refined oil cup. If the soap stock is too soft to drain, pour off several times. Weigh the soap stock immediately to prevent any moisture loss.

Melt the soap stock in water-bath at 75 ± 2°C without stirring for 30 min and then cool in cold water (15°C to 20°C) for 15 min till it is chilled. Decant all possible oil in a tared beaker. Repeat remelting until not more than 1.5 g is recovered. Record the weight of all the nil thus obtained. If oil is difficult to remove due to soft soap stock, use a pipette.

Weigh the refined oil and filter through a specified filter into a clean dry container.

C-6 CALCULATION

Calculate the refining loss by the following methods:

a) Refining loss, percent = $\frac{W_1 - W_2}{5}$

Or

b) Refined loss, percent = $\frac{(A+B)-C}{5}$

where

W_1 = Weight of crude oil, in g,

W_2 = Weight of refined oil, in g,

A = Weight of shop stock, in g;

B = evaporation loss, in g, and

C = Weight of the 20° Be alkali solution added, in g

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This Indian Standard has been developed from Doc No.: FAD 13 (2628).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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Published by BIS, New Delhi